

skin lesions from patients with subacute cutaneous lupus erythematosus

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Objectives: To investigate whether tumour necrosis factor α (TNF α) is expressed in subacute cutaneous lupus erythematosus (SCLE) skin lesions.

Methods: The in situ expression of TNF α in refractory lesional and non-lesional skin biopsy specimens from patients with SCLE was analysed using an immunohistochemical approach. At the time of biopsy these patients were receiving treatment with systemic medications such as antimalarial agents, immunosuppressive drugs, and thalidomide. Expression of TNF α was also evaluated in cutaneous lesions of patients with other inflammatory and neoplastic skin diseases as controls.

Results: The data showed that refractory lesional skin tissue from patients with SCLE displays a strongly positive distribution of TNF α , particularly within the epidermis. No prominent staining was seen in non-lesional skin from the same group of patients or in cutaneous lesions from the control group.

Conclusions: These findings suggest that TNF α is localised and produced by epidermal cells within SCLE skin lesions and support its potential role in the pathogenesis of SCLE. The tissue localisation of TNF α may represent a potential therapeutic target providing a new perspective in the treatment of refractory skin lesions in patients with SCLE.

Many cytokines, including tumour necrosis factor α (TNF α), seem to have a significant role in the inflammatory process, as well as in the regulation of the autoimmune response in systemic lupus erythematosus (SLE).

High serum levels of this cytokine and receptors (sTNFRI and sTNFRII) have been detected in patients with SLE in correlation with disease activity,^{1,2} even during pregnancy.³

It is worth noting that the increased TNF α found in SLE sera is bioactive,⁴ supporting the hypothesis that TNF α contributes to the pathogenesis of some lupus manifestations.⁴ In addition, TNF α has been specifically immunolocalised in kidney sections from patients with SLE and glomerulonephritis⁵ in correlation with histological activity.

TNF α is produced by a variety of cells, including those of the epidermis; in particular, keratinocytes are a potent source of cytokines.⁶

Subacute cutaneous lupus erythematosus (SCLE) is one of the LE-specific skin lesions consisting of non-scarring papulosquamous or annular skin lesions that occur in characteristic photodistribution.⁷ About 75% of SCLE can be managed with conventional topical and systemic treatment; in the remaining 25% of cases skin lesions can be particularly severe and refractory to conventional treatment.

Our study aimed at investigating the in situ expression of TNF α in cutaneous lesions in patients with SCLE.

PATIENTS AND METHODS

Patients and skin biopsies

Upon informed consent, excisional biopsy specimens of lesional and non-lesional sun protected skin were obtained from four patients with SLE (American Rheumatism Association criteria) with specific and diagnostic cutaneous manifestations of SCLE according to Gilliam and Sontheimer's classification.⁷

Detection of autoantibodies

Antinuclear antibodies and anti-double stranded DNA antibodies were detected by indirect immunofluorescence technique, on HEp-2 cells and *Crithidia luciliae*, respectively.

Antibodies against extractable nuclear antigens were tested by immunoblotting technique on cytoplasmic and nuclear cell extracts from Raji cultured cells.

Antibodies directed against SSA/Ro and SSB/La antigens were also tested by enzyme linked immunosorbent assay (ELISA) (INOVA Diagnostics, Inc), following the manufacturer's instructions.

TNF α serum levels

TNF α was measured in the serum of patients with SCLE by chemiluminescence immunoassay using an automated analyser (Immulite, DPC) and anti-TNF α monoclonal antibody (DPC, Biermann GmbH).

Direct immunofluorescence

Cryosections of 4 μ m thickness from lesional and non-lesional skin biopsy specimens were mounted on Polysine glass slides and air dried for 30 minutes. The sections were then stained for 30 minutes in a moist chamber at room temperature, using fluorescein isothiocyanate labelled, rabbit antibodies against human IgG, IgA, IgM, C3, C1q, and C4 (Dako, Glostrup, Denmark). Conjugate dilutions were made in 0.01 M phosphate buffered saline (PBS), pH 7.3, supplemented with 1% bovine serum albumin. After washing in PBS for 30 minutes, the sections were coverslipped under fresh PBS/glycerol (50% vol/vol).

Immunohistochemistry

Detection of TNF α was performed on frozen sections from four samples of active lesions of SCLE, four of normal human skin obtained from sun protected areas of the same patients,

Abbreviations: APAAP, alkaline phosphatase anti-alkaline phosphatase; mAbs, monoclonal antibodies; PBS, phosphate buffered saline; SCLE, subacute cutaneous lupus erythematosus; SLE, systemic lupus erythematosus; TNF α , tumour necrosis factor α .

Table 1 Prominent features at the time of biopsy in our patients affected with SCLE

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
Sex/age (years)	F/28	F/37	F/33	F/49
Disease duration (years)	6	14	14	11
Skin lesion duration (months)	60	170	12	134
Type of skin lesion	Papulosquamous	Papulosquamous	Annular/polycyclic	Annular/polycyclic
Biopsy site:				
Lesional skin	Back	Shoulder	Back	Chest
Non-lesional skin	Gluteus	Gluteus	Gluteus	Gluteus
TNF α staining	Moderate	Moderate	Moderate	Strong
TNF α serum levels (ng/l)*	18.6	13.0	7.3	9.9
ECLAM	6	4	1	2
Clinical manifestations:				
Arthritis	+	–	–	–
Pericarditis	–	–	–	–
Pleuritis	–	–	–	–
Renal disease	–	–	–	–
Haematological disorders	+	+	–	–
Neurological disorders	–	+	–	–
Autoantibodies:				
ANA	+	+	+	+
Anti-dsDNA	–	+	–	–
Anti-La/SSB	–	–	+	–
Anti-Ro/SSA 60 kDa	–	–	+	+
Anti-Ro/SSA 52 kDa	–	–	+	–
Anti-ribosomal P proteins	+	+	–	–
Anti-Sm	–	+	–	–
Prednisone (mg/day)	25	10	5	5
Other treatment	MMF	MMF, HCQ	HCQ, AZA	HCQ
Thalidomide (mg/day)	–	–	–	25

*Reference level 8.1 ng/l.

F, female; ECLAM, European Consensus Lupus Activity Measure; ANA, antinuclear antibodies; MMF, mycophenolate mofetil; HCQ, hydroxychloroquine; AZA, azathioprine.

three samples of mycosis fungoides, three of cutaneous T cell pseudolymphoma, and two of parapsoriasis.

Samples were stained with the following monoclonal antibodies (mAbs): anti-TNF α (clone 68B6A3L1; BioSource Europe, Nivelles, Belgium),⁸ anti-CD3 (clone UCHT1; Dako), anti-CD1a (clone NA1/34; Dako), and anti-CD19 (clone HD37; Dako).

Air dried, acetone fixed, frozen sections were incubated overnight with the mAbs and, after washing, processed with a standard alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. Rabbit antimouse immunoglobulin

(Dako) was applied for 30 minutes and, after washing, the sections were incubated with APAAP complex (Dako) for 30 minutes. Naphthol-AS-MX phosphate together with fast red TR salt were used for the development of alkaline phosphatase. The endogenous alkaline phosphatase was blocked by adding levamisole to the substrate. Sections were counterstained for 5 minutes with Mayer's haematoxylin. Negative controls were performed by omitting the primary mAb on samples or consisted of replacement of the primary antibody with another irrelevant mAb of identical isotype.

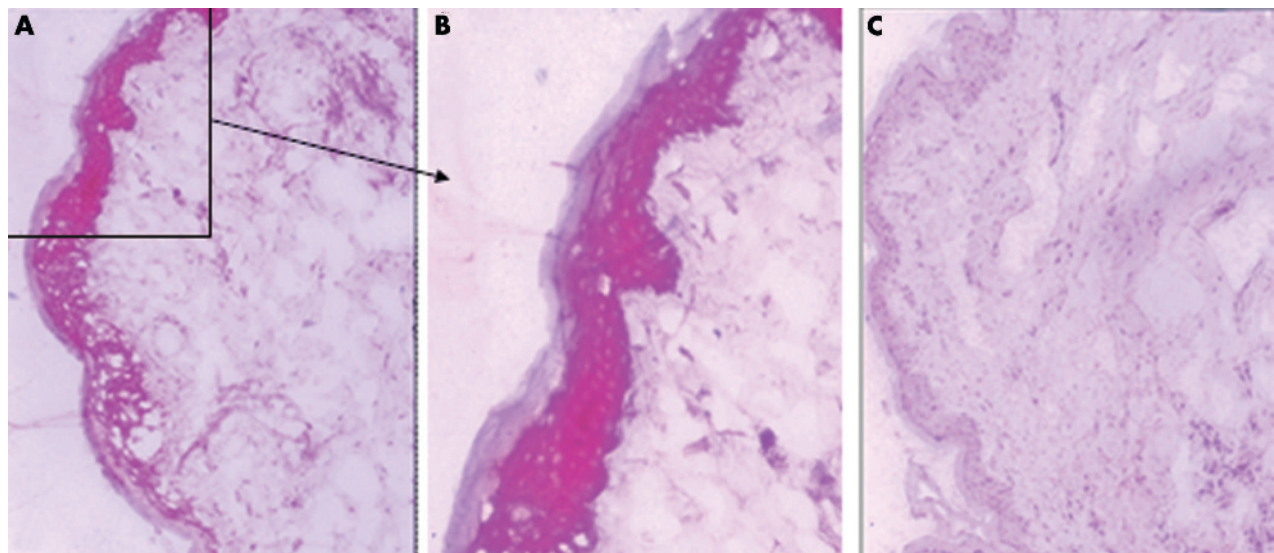


Figure 1 Representative image of strong immunohistochemical staining of TNF α in a case of SCLE (patient No 4). TNF α is clearly expressed in the epidermal compartment of lesional skin (A and B), whereas it is absent in the epidermis of non-sun-exposed skin from the same patient (C). A high power magnification of the positive epidermal areas is shown (B).

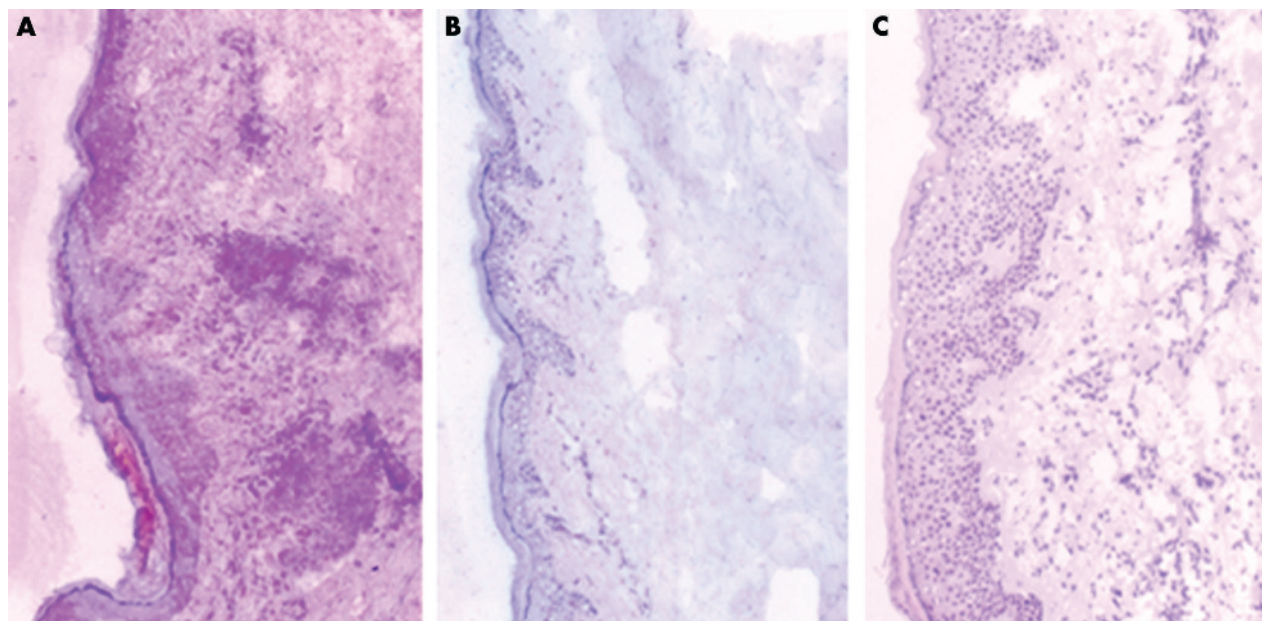


Figure 2 Representative image of moderate staining of TNF α in a case of SCL (patient No 1). In the lesional skin, TNF α is expressed within the epidermis (A) and positive dermal lymphocyte infiltrate can be identified as well (A). The sun protected normal skin from the same patient is negative (B). Representative image of negative TNF α staining in a control case of parapsoriasis (C).

RESULTS

Table 1 summarises the demographic, clinical, and immunological data of the patients at the time of the biopsy.

All patients were treated initially with topical corticosteroids. Because topical treatment did not provide adequate improvement of the skin lesions, patients were treated with conventional systemic treatment, and three of the four also with thalidomide. At the time of biopsy only one patient was still receiving treatment with thalidomide, because the other two patients had stopped treatment owing to the occurrence of peripheral neuropathy. Moreover, at the time of biopsy one patient was not taking antimalarial drugs because they had been withdrawn owing to side effects (itch).

Two independent observers, blindly identified moderate to strong staining of TNF α in the lesional epidermis of the four samples of SCL (table 1, figs 1 and 2). Positive staining was observed on keratinocytes of all layers of the epidermal compartment. In particular, no correlation between TNF α tissue localisation and duration of skin lesions or systemic treatment was seen in any of the patients (table 1). Samples of both the sun protected normal skin from patients with SCL and the pathological skin conditions investigated were negative for TNF α immunostaining.

TNF α serum levels were higher than the reference level in patients Nos 1 and 2, and borderline in patient No 4 (table 1) and the TNF α level seemed to parallel the ECLAM score. No relationship between the TNF α serum levels and their expression in lesional skin tissue was found.

A mild (cases 1 and 2) to moderate (cases 3 and 4) dermal lymphocyte infiltrate was seen in lesional skin of all cases of SCL. The predominant cells at the dermal-epidermal interface were identified as CD3+ T lymphocytes, whereas CD19+ B cells were generally absent. CD1a expression was generally reduced in the epidermal compartment of affected skin of SCL, in comparison with sun protected normal skin from the same patient.

Direct immunofluorescence of lesional skin showed deposition along the dermal-epidermal junction of IgG, IgM, C3, C1q in cases 1 and 2, IgM in case 3, IgG, IgM, and C3 in case 4.

DISCUSSION

It has been demonstrated that there is a strong association of the polymorphism of -308A promoter of TNF α linked to HLA-DR3 with SCL.⁹ Recently, similar findings have been reported also in children affected with cutaneous neonatal lupus, a type of skin lesion which resembles SCL.¹⁰ In addition, in the same study it was shown that TNF α is expressed within the epidermis of lesional skin biopsy specimens from affected children.¹⁰ These findings support the hypothesis of a pathogenetic role of this proinflammatory cytokine in the pathogenesis of cutaneous neonatal lupus.

In our study, we observed that lesional skin of patients with SCL was characterised by an increased expression of TNF α in comparison with normal skin obtained from the same patient and with a small group of inflammatory and neoplastic skin conditions. TNF α immunostaining was mainly localised in the epidermal compartment, suggesting that keratinocytes are the major source of this cytokine during the cutaneous inflammatory process.

A moderate dermal lymphocyte infiltrate was generally detected in lesional skin biopsies investigated, and the characterisation of cell infiltrates showed the presence of CD3+ cells, whereas CD19+ B cells were almost absent. No correlation was found between TNF α immunostaining or CD3+ dermal infiltrates and skin lesions or the duration of systemic treatment.

In addition, CD1a+ cells were reduced in lesional skin biopsy specimens compared with non-lesional skin from the same patient. CD1a is expressed in differentiated antigen presenting cells and monocytes and is used as a marker for the presence of Langerhans' cells in the skin.¹¹ Skin antigen presenting cells (Langerhans' cells) constitute a subset of dendritic cells which appear to have an important role in the initiating events that lead to the T cell mediated immune responses in skin. Our findings indicate that these cells may not have a pivotal role in the immunopathological mechanism involved in the development of cutaneous lesions in SCL.

We found that TNF α serum levels were associated with disease activity, but we did not find a relation between the

level of TNF α in the sera and the intensity of the immunostaining on skin tissue. These findings support the hypothesis that TNF α is locally produced by keratinocytes and could have not leaked from the patient's circulation into inflammatory lesions, binding to TNF receptors locally.

There is strong evidence that thalidomide, a TNF α blocking agent, can be very effective in the treatment of some refractory SCLE. However, the relevant side effects of this drug have to be carefully considered. In fact, thalidomide is highly teratogenic and can induce irreversible peripheral neuropathy.¹²

Recently, an open label study on patients treated with a chimeric anti-TNF α antibody in addition to conventional treatment, showed a clinical improvement in the inflammatory manifestations of the disease, although the development of autoantibodies in those patients has been reported.¹³ The possibility of inducing cutaneous LE during anti-TNF α treatment has to be considered as a possible side effect.¹⁴

The results of our study indicating the presence of TNF α in the epidermal compartment of active lesions of SCLE are consistent with the hypothesis of localised and site-specific contribution of TNF α to the pathogenesis of the skin disease.

Successful treatment with TNF α blockers in a patient with SCLE¹⁵ and our findings suggest that anti-TNF α might be useful in the treatment of selected cases of refractory SCLE.

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